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Electrochemically Active Soluble Mediators from *Shewanella oneidensis*: Relevance to Microbial Fuel Cells and Extracellular Electron Transfer

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Chemotrophic microorganisms harvest energy from their growth substrates through coupled redox reactions that shuttle electrons to terminal electron acceptors. Classic aerobic and anaerobic respiratory chains are well studied and understood. Select bacteria are able to use insoluble metal ions as terminal electron acceptors. The respiration style requires a mechanism that effectively promotes extra-cellular electron transfer to support cell function and growth. One mechanism microbes utilize resembles physical wiring where the microbe grows fine conductive appendages that appear able to allow electron transfer from the cell to the metal ion acceptor[1]. A second approach is the use of soluble mediators such as, quinones, phenazines, and riboflavin, which are able to shuttle electrons from the cell to the terminal acceptor [2]. Understanding electrochemistry of extra-cellular electron transfer is relevant to predicting environmental biogeochemical cycles, as well as in engineering issues for biologically initiated corrosion and the development of microbial fuel cells.

Shewanella oneidensis MR-1 is a gram-negative facultative anaerobe that can use manganese and iron oxides as terminal electron acceptors. Numerous mechanisms appear to be employed by the organism to achieve the phenotype, but numerous questions remain about the fundamental physiology, genetics, and biochemistry relevant to *S. oneidensis* metal reduction [3]. The aim of our program is to evaluate and understand the behavior of strain MR-1 and its derivatives when used as bioelectrocatalysts in microbial fuel cells. The focus in the present work is the identification of secreted mediators that *S. oneidensis* synthesizes in response to various growth conditions and how the mediators may influence strain function in microbial fuel cells. Particular attention was made to mediator synthesis at low pH; theoretical calculations using standard Nernst equations indicate the oxygen reduction reaction potential will shift to enhance fuel cell efficiency with decreases in pH.

S. oneidensis strain MR-1 and the rifampicin-resistant mutant, strain DSP-10, were cultivated at various pH in complex (LB) and defined mineral media at 25°C with gentle agitation (100 rpm). The experiments with microbial fuel cells (MFC) used the previously described mini-MFC module [4], graphite felt was used as the anode material; for the cathode, experiments were done using the equivalent graphite felt or graphite felt coated with platinum nanoparticles. Fuel cell chambers were separated using a gas-permeable polycarbonate membrane. Microbes used in MFC tests were obtained from stationary phase cultures (96 h post inoculation). Electrochemical analysis of culture supernatants was done using a glassy carbon working electrode, a gold counter electrode and a Ag/AgCl reference electrode. Reverse-phase HPLC was used to separate and analyze metabolic compounds from culture supernatants.

MFC power output measurements from the various cell preparations revealed that cell density alone could not account for differences between the tests. Because experiments used planktonic *S. oneidensis*, extra-cellular electron transfer could involve both direct (contact) and indirect (mediated) mechanisms. The results suggest that synthesis and/or excretion of soluble mediators influences electron transfer and in turn, power density. Results from analysis of culture supernatants from media at various pH using cyclic voltammetry (Fig. 1), UV-visible spectroscopy and HPLC was consistent with the hypothesis that differential secretion of redox active compounds occurs in the pH range tested. Cyclic voltammetry confirmed the presence of redox active compounds in culture filtrates (Fig. 1). Media collected from both strains indicated an anodic peak at -650 mV and cathodic peak at -590 mV. The shift of the redox waves for the DSP10 strain filtrate at pH 5 compared to pH 7 (Fig 1B) are indicative of the changed mediator concentration and corresponding inability to catalyze the oxidation of the LB broth.

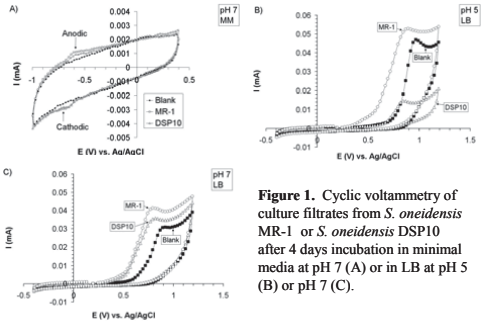

Figure 1. Cyclic voltammetry of culture filtrates from *S. oneidensis* MR-1 or *S. oneidensis* DSP10 after 4 days incubation in minimal media at pH 7 (A) or in LB at pH 5 (B) or pH 7 (C).

The differential signal was corroborated in HPLC analysis of the culture filtrates. A single compound was observed in culture filtrates that showed a changed concentration dependent on medium pH. The excreted compound co-elutes with riboflavin and shares significant identity with the riboflavin UV-vis spectrum (Fig 2). The preliminary results show some insight to differential secretion of redox mediators by *S. oneidensis*. Further experiments will define identity of the putative mediator and confirm whether it is the sole redox active molecule under the experimental conditions. The insight may promote optimal current and power output of MFC.

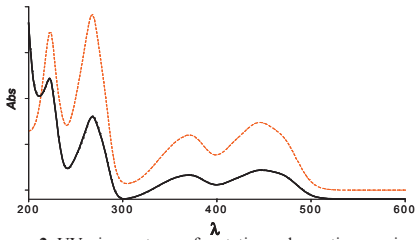

Figure 2. UV-vis spectrum of putative redox active species from *S. oneidensis* culture (solid line) and UV-vis spectrum of riboflavin (red, dashed line).

References:

1. Gorby, Y. & *al., e.* Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* MR-1 and other microorganisms. *Proc. Natl. Acad. Sci. USA* **103**, 11358-11363 (2006).
2. Stamms, A. J. M. *et al.* Exocellular electron transfer in anaerobic microbial communities. *Environ. Microbiol.* **8**, 371-382 (2006).
3. Bretschger, O. et al. Current Production and Metal Oxide Reduction by *Shewanella oneidensis* MR-1 Wild Type and Mutants. *Appl. Environ. Microbiol.* **73**, 7003-7012 (2007).
4. Ringeisen, B. R. *et al.* High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSP10. *Environ. Sci. Technol.* **40**, 2629-2634 (2006).

11:00 **189** Evidence of Structural Changes in the Positive Electrode of Nickel Batteries during Normal Charge/Discharge Operation - M. Casas-Cabanas (EMAT), J. Canales-Vazquez (Institut de Ciencia de Materials de Barcelona (ICMAB)), J. Rodriguez-Carvajal (Institut Max Von Laue-Paul Langevin (ILL)) and M. Palacin (Institut de Ciencia de Materials de Barcelona (ICMAB))

11:20 **190** High-Rate Alkaline CuO Cathodes - S. W. Donne and T. Jones (University of Newcastle)

Electrolyte Membranes
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14:00 **191** Direct Ethanol Fuel Cells Using Alkaline Anion Exchange Membrane - P. Lim, G. Li and C. Wang (Penn State University)

14:20 **192** Phosphonium and Ammonium Based Membranes for Carbonate and Alkaline DMFC - J. Zhou, J. Vega, W. Mustain and P. Kohl (Georgia Institute of Technology)

14:40 **193** Some Approaches to Form Triple Phase Boundary on Oxygen Cathode of Anion Exchange Membrane Fuel Cells - Z. Ogumi, K. Nishio, K. Miyazaki, T. Abe, Y. Iriyama (Kyoto University), H. Nakanishi and S. Matsumoto (Toyota Motor Corporation)

15:00 **194** A Dusty Fluid Model to Predict Hydroxyl Ion Conductivity in Alkaline Anion Exchange Membranes - K. Grew and W. Chiu (University of Connecticut)

Alkaline Supercapacitors
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15:40 **195** Co_xNi_{1-x} Oxides Thin Films for Supercapacitor Applications - V. Gupta, S. Gupta and N. Miura (Kyushu University)

16:00 **196** Capacitance Fade in Alkaline Manganese Oxide Supercapacitor Electrodes - S. W. Donne, B. Jones (University of Newcastle) and T. Hollenkamp (CSIRO Division of Energy Technology)

16:20 **197** Multifunctional MnO_2-Carbon Nanoarchitectures as High-Performance Electrode Structures for Alkaline-Electrolyte Batteries and Electrochemical Capacitors - J. W. Long, A. Fischer, M. Saunders, K. Pettigrew and D. Rolison (Naval Research Laboratory)

16:40 **198** Electrochemical Properties of Ni-Co Binary Oxide / Carbon Composite for Electrochemical Capacitor Application - J. Kim and K. Kim (Yonsei University)

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Energy Technology / Physical and Analytical Electrochemistry / Organic and Biological Electrochemistry

Room 207, 200 Level, Phoenix Convention Center

Microbial Fuel Cell Fundamentals
Co-Chairs: F. Mansfeld and S. Minteer

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08:20 **231** Effect of Biofilm Properties on the Electrochemical Performance of Microbial Fuel Cells - R. P. Ramasamy, S. RedCloud-Owen, Z. Ren, J. Regan and M. Mench (Pennsylvania State University)

08:40 **232** Direct Electrochemical Behavior of Evolved Escherichia Coli Cells in Microbial Fuel Cells - C. Li, Y. Qiao and S. Bao (Nanyang Technological University)

09:00 **233** High Throughput Screening Array for Electrochemically Active Bacteria (EAB) using Voltage Detection - J. C. Biffinger (US Naval Research Lab), M. Ribbens, S. Finkel, K. Nealson (University of Southern California) and B. Ringeisen (US Naval Research Lab)

09:20 **234** Electrical Transport in Bacterial Nanowires: Implications for the Microbial-Inorganic Interface in Microbial Fuel Cells - M. Y. El-Naggar (University of Southern California), Y. A. Gorby (J. Craig Venter Institute) and K. Nealson (University of Southern California)

09:40 Intermission (20 Minutes)

10:00 **235** Using the Microbial Fuel Cell to Reveal Patterns of Microbial Metabolism, Biochemistry, and Behavior - K. Nealson and O. Bretschger (University of Southern California)

10:40 **236** Electrochemical Characterization of Mixed Species Biofilms Grown in Microbial Fuel Cells - E. Marsili, C. Harrington (University of Minnesota), T. Shimotori (Microbial Fuel Cell Technologies, LLC), R. Hozalski, T. LaPara and D. Bond (University of Minnesota)

11:00 **237** From Fermentation Waste to H_2: GeoChip-based Analysis of Microbial Community Structure and Functions in Bio-electrochemically Assisted Microbial Reactor - A. Wang (Harbin Institute of Technology), B. Logan (Penn State University), J. Zhou (University of Oklahoma) and J. Tiedje (Michigan State University)

11:20 **238** Electrical Wiring of Living Bacterial Cells Using Flexible Osmium-Redox Polymers - L. Gorton, V. Coman, T. Gustavsson and C. Hägerhäll (Lund University)

11:40 **239** Phosphate and Bicarbonate Buffers as Proton Carriers Inside the Biofilm of Anode-Respiring Bacteria in Microbial Fuel Cells - C. I. Torres, A. Kato Marcus and B. Rittmann (Arizona State University)

Microbial Fuel Cell Systems
Co-Chairs: K. Nealson and P. Atanassov

14:00 **240** Two Chambers Biofuel Cell of Producing Power and Bio-hydrogen from Treating Organic Wastewater - L. Wang (Shenyang Institute of Chemical Technology) and J. Wu (Shenyang Insitute of Chemical Technology)

14:20 **241** Electricity Generation Using Single Chamber Microbial Fuel Cells Fed by Wastewater - J. Zuo, Q. Deng, L. Cui (Tsinghua University), Y. Sun (Beijing Normal University) and Y. Dang (Tsinghua University)